FY2001 Investigational Report:

Health Monitoring of Hatchery and Natural Fall-run Chinook Salmon Juveniles in the San Joaquin River and Tributaries, April – June 2001

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Summary

We conducted a survey of the health and physiological condition of juvenile fall-run Chinook salmon in the San Joaquin River and three tributaries (Stanislaus, Tuolumne and Merced Rivers) during the spring of 2001. This was an expansion of a study in the San Joaquin River and Delta conducted during the spring of 2000. The incidence of pathogen infection was higher in this study than in the 2000 survey. *Renibacterium salmoninarum* (the causative agent of Bacterial Kidney Disease) was detected in naturally produced fall Chinook juveniles from the Stanislaus, Tuolumne and mainstem San Joaquin Rivers. Proliferative kidney disease (PKD) was detected in both natural and hatchery fall Chinook juveniles from the Merced and mainstem San Joaquin Rivers. Proliferative kidney disease could be a significant contributor to mortality in natural smolts. We recommend monitoring the incidence of BKD and PKD in the San Joaquin River basin to determine the impact of these diseases to the natural fish population. Physiological measurements were not significantly different than expected from juveniles undergoing smoltification. Measures of energy stores and fish condition declined while smoltification progressed at most sites. Fish underwent smoltification earlier in the Tuolumne River than in the Stanislaus or Merced Rivers

Introduction

Declining Chinook salmon (*Oncorhynchus tshawytscha*) populations in California's Central Valley are prompting an intense restoration effort of this valuable resource and a key element of the state's aquatic biodiversity. Health and fitness of juvenile salmon out-migrants ("smolts") are major determinates of their performance and survival. Contaminants and elevated water temperature are stressors with the potential for immunosuppressive effects. Infectious disease can influence survival due to both direct mortality and reduce physical performance (predator avoidance, saltwater adaptation, etc.). Knowing the disease status of fish populations allows managers to make informed decisions when moving fish and equipment within or between water bodies. While hatchery – wild fish interaction is a controversial topic in natural resource management, a comparison of the pathogens present in both populations is needed to either support or refute the charge that hatchery fish spread disease to natural populations. The criteria used to define a quality hatchery fish is being reviewed and debated among hatchery and fish biologists. It is important to profile the physiological condition of natural populations compared with hatchery fish.

Rich and Loudermilk (1991) performed a physiological survey, emphasizing smolt development, that included hatchery and naturally produced juvenile San Joaquin River Fall Chinook Salmon. During the spring of 2000, the California-Nevada Fish Health Center conducted a health and physiological condition survey in the San Joaquin River and Delta that looked at both hatchery and natural origin smolts (Nichols et al. 2001). These studies provided some data on the health and physiological development of fish in the mainstem San Joaquin including hatchery verses natural origin comparisons. In these studies, natural origin fish appeared similar to their hatchery origin cohorts.

This study was an extension of the 2000 health survey and examines naturally produced smolts from three tributaries of the San Joaquin River (Stanislaus, Tuolumne and Merced Rivers) and hatchery origin smolts from Merced River Hatchery (MRH). Additionally, mixed origin smolts from the San Joaquin River below the three tributaries

were collected for examination. We looked for trends in disease status, smolt development and condition indices between sites and over time at sites.

Methods

Fish collection

Fall-run Chinook salmon were collected in conjunction with ongoing juvenile salmonid monitoring in the San Joaquin River basin. Sample sites included: MRH operated by the California Department of Fish and Game (CDFG); Merced River at a rotary screw trap (RST) operated by Natural Resource Scientists, Inc; Tuolumne River at a RST operated by the CDFG; Stanislaus River at a RST operated by SP Cramer and Associates, Inc; and mainstem San Joaquin River from a Kodiak trawl operated by the CDFG (Figure 1). Sampling occurred from April 9 – June 7, 2001. If individual catches at a given site were poor, multiple collections for up to an 8-day period were lumped to form one sample, and the day on which a majority of fish were collected was designated the sample date.

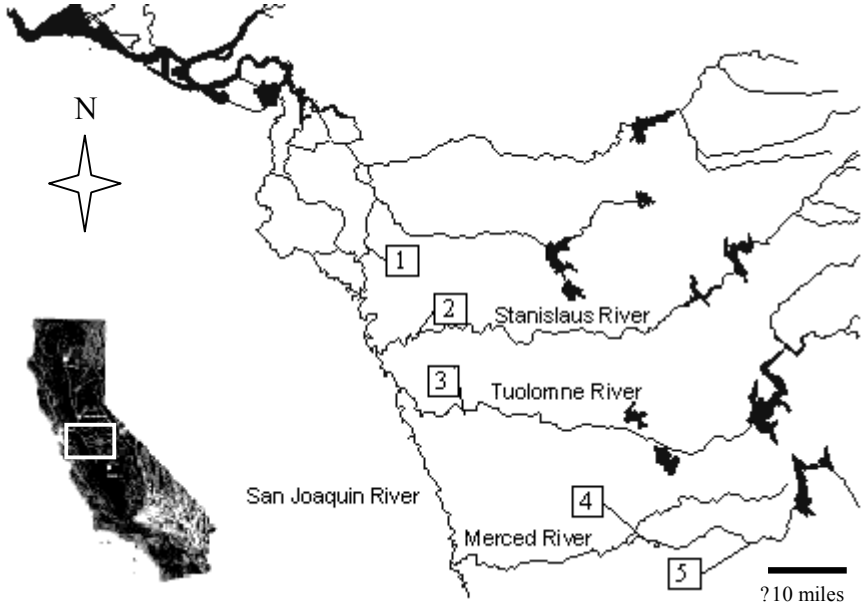

Figure 1. Sites sampled in the San Joaquin River basin April-June 2001. Sites included: mainstem San Joaquin River (1), Stanislaus River (2), Tuolumne River (3), Merced River (4), and Merced River Hatchery (5).

Most MRH origin fish were released into the Merced or mainstem San Joaquin, but small groups of marked MRH fish were released into the Tuolumne and Stanislaus for other studies. Smolts released from MRH were 100% marked with either an adipose fin clip or a fin dye mark prior to May 11. All fish without detectable marks during this period were considered of naturally spawned origin ("natural"). Marked fish were avoided in our sampling prior to May 11. After May 11, identification of natural smolts was not attempted, as unmarked MRH fish were present.

Hatchery origin fish were sampled at MRH prior to release. The hatchery was located at river mile (RM) 52 on the Merced River about 170 miles from the mouth of the San Joaquin River (SJRM). This site was sampled on April 19 approximately 1 week prior to the first large scale releases. Four groups of 15 fish per raceway were collected by dip net representing the range of fish sizes (ages) present at the hatchery.

Natural smolts were sampled from the Merced, Tuolumne and Stanislaus Rivers. The Merced River site was located upstream of the HWY 59 bridge at RM 37 (SJRM 155). The Tuolumne River site was located upstream of the Shiloh bridge at RM 13 (SJRM 96). The Stanislaus River site was located near Caswell State Park at RM 9 (SJRM 84). These sites were sampled on April 18 and May 8 (Stanislaus sampled May 8 and 9). The April sample was timed to capture fish that were of a minimum size to provide enough tissue for the laboratory assays (about 70mm fork length). While the sample date was chosen to achieve a minimum size, no fish were excluded due to size during sampling. The May sample date was selected as the last week it was likely to capture a 30 fish sample. Fish were removed from the RST during the first trap check in the morning and first processed by the cooperator at each site. Up to thirty live fish were then transferred to a live well for necropsy on site.

Hatchery and natural smolts were sampled from the mainstem San Joaquin River. Sampling was performed downstream of all tributary sites in a reach with some tidal influence and near the upper boundary of the San Joaquin River Delta. The area trawled was below Mossdale Crossing at RM 54. This site was sampled on April 10, April 17, May 1, May 30 and June 7. The April and early May dates were chosen to correspond with tributary sampling, and fish were of natural origin smolts. The late May/early June period was chosen to target peak ATPase values observed in previous studies (Rich and Loudermilk 1991, Nichols et al. 2001), and smolts were a mix of hatchery and natural origin. Fish were in the trawl for up to 20 minutes and handled by the CDFG crew prior to our sampling. Necropsy was performed between trawls, and multiple trawls were included in each sample group.

Water temperature and flows

Flow and temperature data were obtained from the California Data Exchange Center website (http://cdec.water.ca.gov). Water year classified as: wet, above normal, below normal, dry, or critical based on the San Joaquin Valley Water Year Hydrological Classification. Flow data (mean daily) sites included: San Joaquin River at Vernalis (VNS), Stanislaus River at Orange Blossom Bridge (OBB), Tuolumne River at Modesto (MOD), and Merced River near Stevinson (MST). Temperature data (hourly) sites included: San Joaquin river at Vernalis (VER), Stanislaus River before confluence of San Joaquin (SBC), Tuolumne River at Modesto (MOD), and Merced River near Stevinson (MST).

Necropsy

Fish were examined and tissue samples were collected at each site. Fish were euthanized with an overdose of anesthetic in small groups, measured for fork length (±1mm), and weighed (±0.1g). Any gross abnormalities of the gill, skin or eyes were scored, the quantity of visceral fat was scored (Appendix 1) and any organ abnormalities

or signs of disease were recorded. Tissue samples were collected for pathogen and physiological laboratory assays.

Assays

Bacteria – A sample of kidney tissue was taken aseptically from each fish and inoculated onto individually numbered brain heart infusion agar slant tubes. Isolates showing growth within 3 days were screened for bacterial fish pathogens by standard microscopic and biochemical tests (Lasee 1995). Bacterial isolates that were ubiquitous in freshwater (all isolates except potential *Aeromonas salmonicida*, *Yersinia ruckeri* or *Edwardsiella tarda*) and without associated clinical signs of infection were identified to general group. The methods used would not have detected the *Flavobacterium* group including *F. columnare*.

Virus –Two to four-fish pooled samples of kidney were assayed for virus on both EPC and CHSE-214 cell lines for 15 days at 15°C by standard tissue culture methods (Thoesen 1994).

Rs-DFAT – A direct fluorescent-antibody technique (DFAT) for *Renibacterium salmoninarum* was performed on a digest preparation of the two-fish pooled kidney tissue pellet left over after the viral assay. The digest was performed by adding 1.0 mL of 0.25% trypsin solution (pH adjusted to 8.5 with NaOH) to each pellet sample, mixing, digesting for 1 hr in a 50°C water bath, and centrifuging the sample at 10,000g for 20 min. The supernatant was discarded and duplicate smears made from the pellet. The slides were fixed by both heat and then a 5 min. immersion in absolute methanol. They were stained with a polyclonal fluorescent antibody and examined at 600x magnification on an Olympus BH2 fluorescence microscope.

Histopathology –The viscera (intestinal tract, pyloric caeca, heart, liver and spleen) and posterior kidney were rapidly removed from the fish and immediately fixed in Prefer fixative (Anatech, LTD), processed for 5 μm paraffin sections and stained with hematoxylin and eosin (Humason 1979). All tissues for a given fish were placed on one slide and identified by a unique code number. Each slide was examined at both low (40X) and high magnification (400X). Infections of the myxozoan parasite *Tetracapsula bryosalmonae* (causative agent for Proliferative Kidney Disease) were rated as early (few to moderate parasites without significant inflammatory response) or clinical (large numbers of parasites and/or associated inflammation). The presence and extent of the eosinophilic granular cell layer in the lamina propria region of the intestine or pyloric ceca was rated as 0 (no traceable layer), 1 (zone 1-2 cells thick) or 2 (>2 cells thick).

Mc-digest – Screening for *Myxobolus cerebralis* was done by pepsin/trypsin digest of cranial elements and examination by phase contrast microscopy at 400x (Thoesen 1994).

Muscle lipid - A cross section of the caudal peduncle (0.1 – 1.1 g) was dissected, placed into a pre-weighed glass tube with cap and frozen for later analysis. Muscle lipid was extracted using a modification of the Bligh and Dryer (1959) chloroform/methanol method and expressed as a percent of wet weight.

ATPase - Gill Na^+, K^+-Adenosine Triphosphatase activity (ATPase) was assayed by the method of McCormick and Bern (1989). Briefly, gill lamellae were dissected and frozen in sucrose-EDTA-Imidazole (SEI) buffer on dry ice. The sample was later homogenized, centrifuged and the pellet sonicated prior to the assay. ATPase activity was

determined by the decrease over time in optical density (340 nm) as NADH is converted to NAD+. This activity was reported as μmole ADP/mg protein/hour as 1 mole of NAD is produced for each mole of ADP generated in the reaction. Gill Na-K-ATPase activity is correlated with osmoregulatory ability in saltwater and is located in the chloride cells of the lamellae. This enzyme system transports salts from the fish against the concentration gradient to the saltwater.

Plasma lysozyme – The lysozyme activity (mOD/minute/10 μl plasma) was determined from blood plasma samples frozen on dry ice, stored at -70°C, and later assayed by the turbidimetric method described by Ellis (1990). The assay described the activity of the samples as the decrease in optical density when a *Micrococcus lysodeikticus* suspension was lysed by the lysozyme in the sample. Both the number and activation state of phagocytes is correlated with plasma lysozyme activity (Robertsen et al. 1994).

Data analysis

Abnormality scores, visceral fat scores, Fulton condition factor ($K=\text{weight(g)}/\text{FL(mm)}^3 \times 10^5$), and lab results for pathogen and physiological assays were tracked to individual fish using a computer database. Statistical analysis was performed using Sigma Stat 2.0. Due to the bimodal nature of much of the data (parr/smolt or diseased/healthy within the sample) non-parametric methods were chosen for analysis. Rank sum test, ANOVA on ranks, and Spearman rank order correlation were used for analysis.

Results

Water temperature and flows

The California Department of Water Resources rated 2001 as a "dry" year in the San Joaquin Valley. Flows were heavily manipulated during the sample period. In mid-April, mainstem San Joaquin River flows increased from near 2000 to over 4000 cubic feet per second (CFS). In the tributaries, flows (Stanislaus, Tuolumne and Merced Rivers) increased by 100 to 300%. This flow regime continued until mid-May when tributary flows were reduced and mainstem San Joaquin flows were cut back to around 2000 CFS. A typical late spring pattern of gradually decreasing flows occurred over the last few weeks of this study. Water temperatures generally increased during the sample period. Water temperatures in the Tuolumne, Merced and San Joaquin Rivers increased sharply in mid May exceeding the upper incipient lethal temperature (UILT) of 23°C predicted for fish migrating through the Sacramento-San Joaquin Delta (Baker et al. 1995) on May 21. Maximum water temperatures occurred in the Tuolumne (28°C) and San Joaquin (26°C) on May 31 and in the Stanislaus (21°C) and Merced (28°C) on June 1.

Observed abnormalities and clinical signs of disease

The most common abnormality was scale loss observed in 74 of 222 fish (33%). Heavy scale loss was common in fish sampled from the San Joaquin site and was attributed to sampling by trawl. Minor scale loss at several other sites was likely due to the deciduous nature of salmonid scales during smoltification and handling prior to examination. Gill abnormalities were seen in seven of 222 fish (3%). Pale gills were seen in four of 18 fish (22%) from the May Tuolumne sample. No pathogens were

detected in these fish and the abnormality may have been due to extensive handling. Swollen kidneys or enlarged spleens were observed in six of 222 fish (3%) examined during this study. Five of 30 fish (17%) examined in the May Merced sample were observed to have swollen kidneys and spleens. This is a classic clinical sign of Proliferative Kidney Disease (PKD), which was common in the Merced River (see Pathogens below).

Pathogens

The myxozoan parasite *Tetracapsula bryosalmonae*, which causes PKD, was detected in 25 of 90 (28%) histological sections of posterior kidney from individual fish (Figure 2). Various stages of the infection were observed in the study ranging from a few parasites to heavy infections with severe kidney damage. Natural fish from the Merced, Tuolumne and San Joaquin Rivers were infected with *T. bryosalmonae*. The highest prevalence of *T. bryosalmonae* infections was in natural smolts from the Merced River (90-100%). Infections were also seen in 20% of MRH fish and 40% of the mixed hatchery/natural San Joaquin sample.

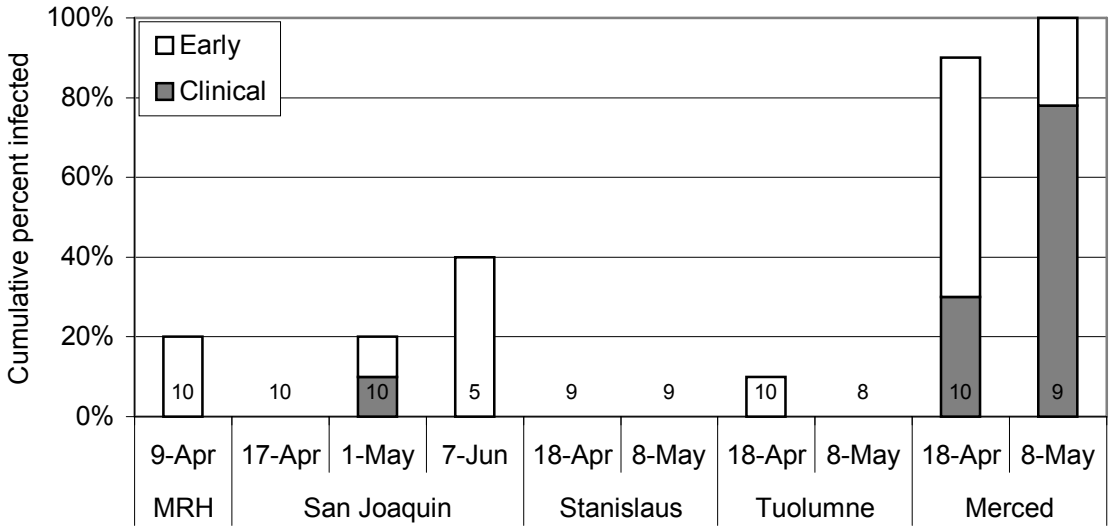

Figure 2. Incidence of *Tetracapsula bryosalmonae* infection (the cause of Proliferative Kidney Disease) in kidney tissue of juvenile Chinook salmon from the Merced River Hatchery (MRH), San Joaquin, Stanislaus, Tuolumne, and Merced Rivers. Typical early stage infections (Early) exhibit relatively few parasites and no associated lesions. Clinical infections (Clinical) are characterized by heavy infections of the parasite with associated lesions likely impairing kidney function. Presented as cumulative percent Early + Clinical and number of samples at each site.

Tetracapsula bryosalmonae (formerly designated PKX) has been described in MRH fish since the 1980's (Hedrick et al. 1986), and review of archived histological samples have shown the disease in California since at least 1966 (Hedrick et al. 1985). Infection with this parasite does not necessarily lead to the death of the animal. Hedrick and Aronstien (1987) found that over 90% of *T. bryosalmonae* infected juvenile Chinook

salmon transferred to full strength seawater were able to recover under laboratory conditions. Survival in the river is likely much lower due to the anemia, kidney dysfunction and immune suppression linked to PKD (Angelidis et al. 1987, Hedrick and Aronstien 1987). This disease compromises the fish's performance in many areas (swimming, salt water entry, disease resistance) and decreases its potential for survival during the hazardous emigration journey. Given the 90+% infection rate we observed, this disease has the potential to be a significant contributor to mortality of natural Merced River Fall Chinook Salmon smolts.

During the spring 2000 survey of natural and hatchery smolts in the San Joaquin River, two of 96 fish (2%) were *T. bryosalmonae* infected (Nichols et al. 2001). This difference between years was possibly due to environmental conditions. There was an "above normal" water year during 2000 and a "dry" year during 2001. During 2000 the San Joaquin River maximum water temperatures reached, but did not exceed the 23°C UILT during the migration period. Infection is dependent on the presence of the infectious stage in the water (Foott and Hedrick 1987), and disease progression is faster at higher water temperatures (Ferguson 1981). The small number of infected fish in the 2000 study suggested a lower concentration of the infectious stage of the parasite. Poor production of the infectious stage in the second (bryozoan) host and/or a dilution effect of higher flows may have limited infection of fish. The geographic distribution of PKD and impact on survival of natural salmonid populations needs further study.

Renibacterium salmoninarum was detected by DFAT in 11 of 112 (2%) pooled (235 total fish) kidney samples (Figure 3). These infections were found in natural smolts sampled from the San Joaquin, Stanislaus, and Tuolumne in April, but were not found in the Merced smolts or in smolts from any site during May. This bacterium was not seen in fish from MRH. The mixed hatchery/natural smolts sampled from the San Joaquin during early June were not screened for this pathogen. No gross clinical signs of Bacterial Kidney Disease (BKD) were seen in any of the fish examined. These low-level infections might have remained active after the salmon entered the ocean phase of their life cycle (Banner et al. 1986) and manifested as clinical BKD due to any number of stressors the fish encountered. Alternately, the fish may well have continued as carriers with low-level infections. Further study is needed to determine the prevalence of infection in the smolt and returning adult populations, and the risk BKD poses to the natural salmonid population.

Bacteria were cultured from 57 of 237 (24%) individual fish, and no primary fish pathogens (*Aeromonas salmonicida*, *Yersinia ruckeri* or *Edwardsiella tarda*) were identified (Table 1). There were no signs of infection or disease associated with these isolates. It was not uncommon for fish to have chronic sub-clinical infections of ubiquitous aquatic bacteria. No correlation was seen between opportunistic bacterial infection and the infections mentioned above (PKD and BKD), but contamination due to sampling conditions at some sites (high wind), may have masked any relationship.

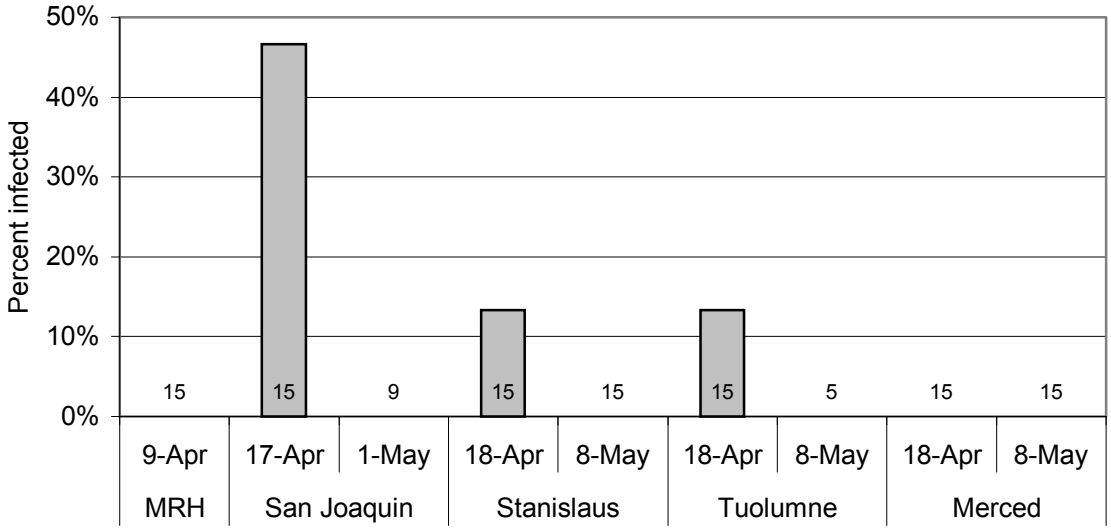

Figure 3. Incidence of *Renibacterium salmoninarum* infection (the cause of Bacterial Kidney Disease) in kidney tissue of juvenile Chinook salmon examined from the Merced River Hatchery (MRH), San Joaquin, Stanislaus, Tuolumne, and Merced Rivers. Number of samples (2-4 fish pools) is given at the bottom of each column.

Table 1. Bacteria groups cultured from kidney tissues of 237 individual juvenile Chinook salmon from the Merced River Hatchery, San Joaquin, Stanislaus, Tuolumne, and Merced Rivers. Further screening of these isolates for *Aeromonas salmonicida, Edwardsiella tarda* and *Yersinia ruckeri* was performed, and these pathogens were not found. No disease signs were associated with these isolates and they were either chronic infections of ubiquitous aquatic bacteria or contaminates due to field sampling condions.

Isolate	Incidence	Percent
Aeromonas/Pseudomonas complex	31	13.1%
Bacillus sp.	5	2.1%
Enterobacteriaceae (family)	6	2.5%
Micrococcus sp.	5	2.1%
Staphylococcus sp.	3	1.3%
Streptococcus sp.	1	0.4%
Other non-pathogens (unidentified)	11	4.6%
Total	57[a] / 237	24%

[a] The total differs from sum of individuals due to mixed isolates in 5 samples.

No virus was detected by tissue culture of 112 pooled kidney samples (235 total fish). These findings are consistent with natural fall-run Chinook salmon work done previously in the San Joaquin River basin (Nichols et al. 2001).

Myxobolus cerebralis (the causative agent of Whirling Disease) was not detected in 20 pooled samples of natural fish (194 total fish). This parasite has been detected previously in rainbow trout (*Oncorhynchus mykiss*) from the Stanislaus and Mokelumne Rivers of the San Joaquin River basin (Horsch 1987, Modin 1998).

Lysozyme

Significant differences in plasma lysozyme activity levels were seen between sample groups in April (Figure 4A) and May (Figure 4B) and at each site over time (Figure 4C). Levels were highest among natural smolts from the Tuolumne in April and the Merced in May. MRH smolt median activity levels were higher than natural smolts in the San Joaquin, Stanislaus and Merced during April. The mixed origin smolts from the San Joaquin taken in June were not significantly different than smolts from the previous two San Joaquin sample dates.

Individual fish with lysozyme activity levels in the upper quartile for all fish from all sites combined (activity >24) were considered elevated. Elevated lysozyme activity levels were seen in natural smolts from the Tuolumne (6 of 15) and Merced (1 of 14) during April and the San Joaquin (1 of 13) and Merced (10 of 15) during May. Elevated activity levels were seen in 11 of 15 smolts sampled at MRH during April. One of the 6 smolts from the San Joaquin site during June had elevated levels. These elevated activity levels were likely responses to pathogens particularly in MRH, Merced River and San Joaquin River fish where PKD was prevalent.

This assay was particularly indicative of the inflammatory response due to PKD. Fish with clinical PKD tended to have elevated lysozyme activity levels. Sixty-eight individual fish were assayed for both lysozyme activities and *T. bryosalmonae* infection ratings. Elevated lysozyme levels were seen in five of 44 (11%) fish with no detected *T. bryosalmonae*, two of 13 (15%) "early" stage and nine of 11 (82%) "clinical" stage fish.

The lysozyme assay did not appear to correlate with *Rs*-DFAT results. Six of the *Rs*-DFAT positive samples (2-pooled) had corresponding lysozyme activity levels. Lysozyme activities ranged from 10 to 25 in these fish, with one slightly elevated sample from the Tuolumne in April. As the *Rs*-DFAT samples were two-pooled, it was impossible to know which or if both fish in the pool were *Rs* infected, and pooling may have clouded any correlation that was present.

Fork length

Significant differences in lengths were seen between sample groups in April (Figure 5A), May (Figure 5B) and at each site over time (Figure 5C). During both April and May, natural smolts from the Tuolumne were larger than smolts from the Merced or Stanislaus. Comparisons between natural and MRH fish were likely not valid due to differences in the sampling methods.

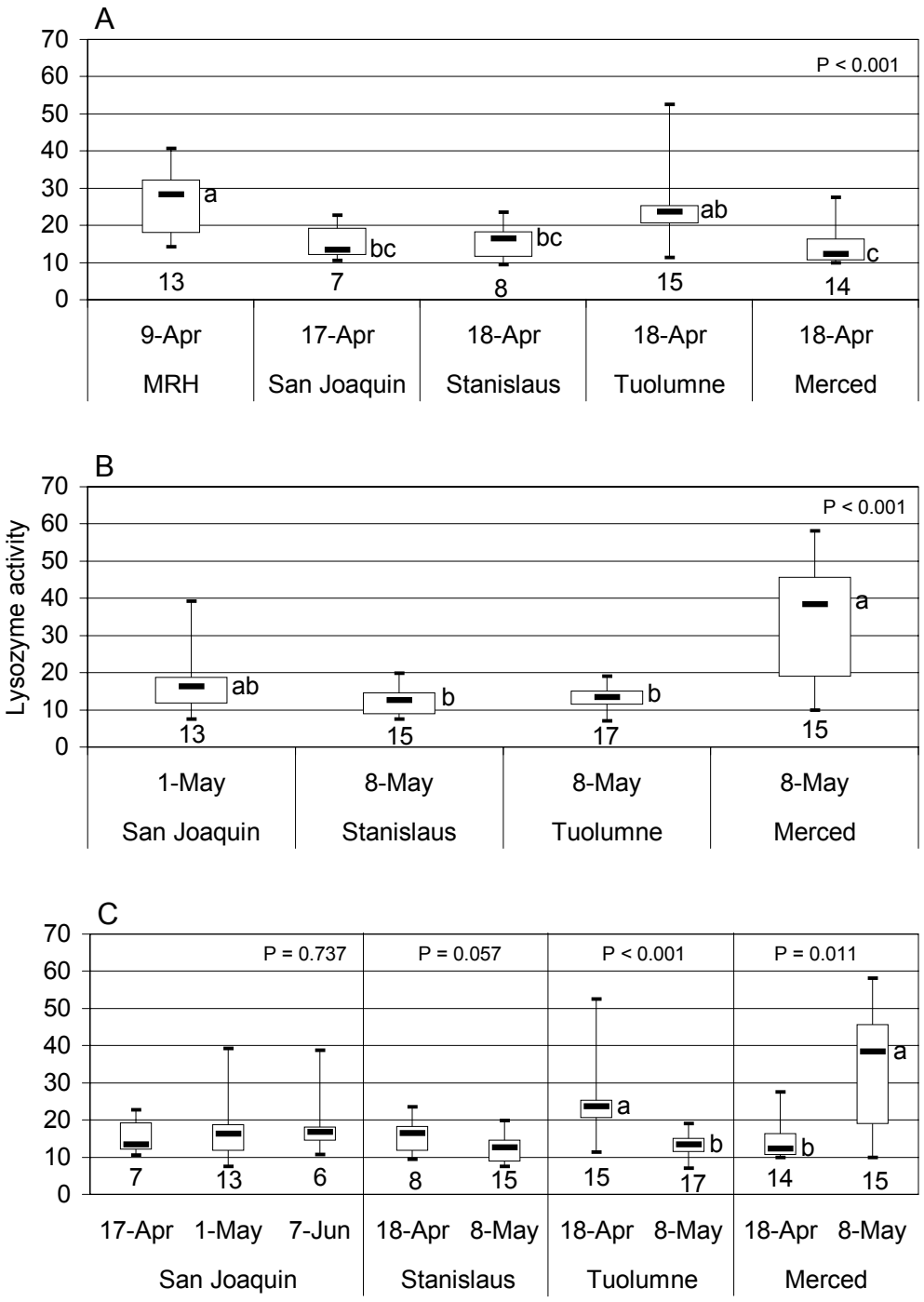

Figure 4. Plasma lysozyme activity (mOD/min/10µl) for juvenile Chinook salmon from the Merced River Hatchery (MRH), San Joaquin, Stanislaus, Tuolumne and Merced Rivers. Data is given as Median, 25%-75% (box), range (whiskers), and sample size. Statistical analysis (ANOVA on ranks or Rank Sum Test) is made between sites for April (A) and May (B) and at each site over time (C). Groups that are significantly different (P<0.05) have no letters in common.

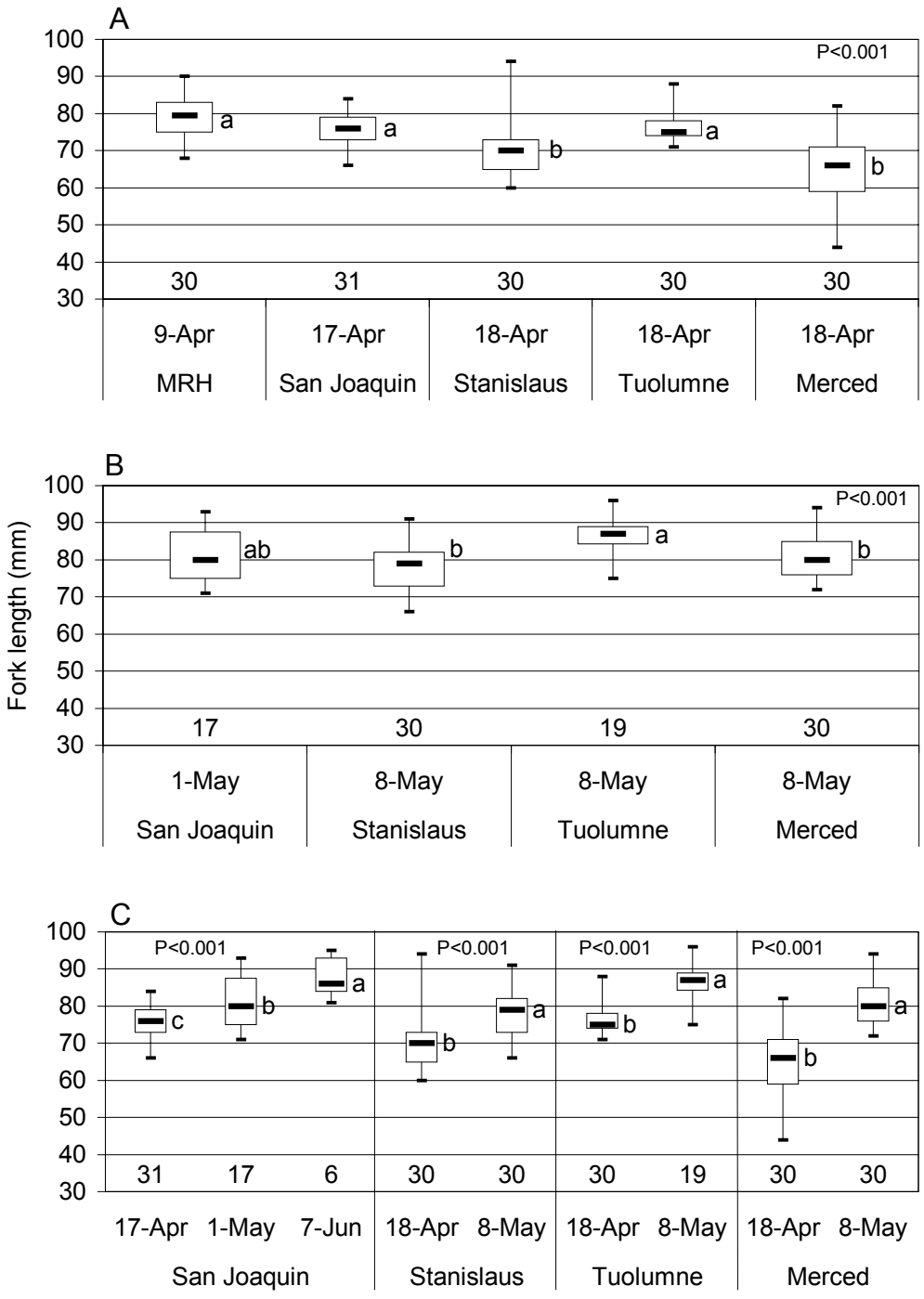

Figure 5. Fork length of juvenile Chinook salmon examined from Merced River Hatchery (MRH), San Joaquin, Stanislaus, Tuolumne and Merced Rivers. Data is given as Median, 25%-75% (box), range (whiskers), and sample size. Statistical analysis (ANOVA on ranks or Rank Sum Test) is made between sites for April (A) and May (B) and at each site over time (C). Groups that are significantly different (P<0.05) have no letters in common.

Condition factor

Significant differences in condition factors were observed between sample groups during April (Figure 6A) and May (Figure 6B) and at each site over time (Figure 6C). Among natural fish, condition factor was variable. The Merced fish were the lowest in April, but were among the highest in May. Between April and May decreasing condition factors were seen in the Stanislaus and San Joaquin smolts while the condition factor in Tuolumne smolts did not change significantly. The highest observed condition factors were in MRH fish. The mixed origin smolts from the San Joaquin in June had a condition factor consistent with that observed in natural smolts.

Given the high-energy cost of the smoltification process we expected condition factors to decline during the study period. Increased condition factor in smolts from the Merced during May could have been caused by kidney dysfunction due to PKD or have been an indication of favorable rearing conditions later in the season.

Muscle lipid and visceral fat observations

Significant differences in percent muscle lipid were noted between samples groups in April (Figure 7A) and May (Figure 7B) and at each site over time (Figure 7C). In natural smolts, the highest levels were seen from the Tuolumne during April and the Merced during May. The lowest values were seen in the Stanislaus smolts during both months. Probably due to their high-energy diet, fish sampled at MRH had the highest lipid levels of all sample groups. Lipid levels in the mixed origin smolts from the San Joaquin during June had similar values to those observed in the natural fish.

Like condition factor, we expected lipid levels to decline over time due to smoltification. Lipid levels likely decreased in hatchery fish after they were released from MRH, but we failed to sample any marked MRH origin fish after release. The only sample sites with smolts indicating a significant decline in lipid levels from April to May were from the Tuolumne where there was no corresponding change in condition factor. The high lipid content in Tuolumne and Merced smolts were possible evidence of an abundant food supply in those systems or that a few hatchery fish were included in those samples despite our efforts to exclude all marked (hatchery) fish.

There was good agreement between visceral fat observations (data not presented) and muscle lipid measurements ($P<0.001$, $n = 122$, Spearman Rank Order Correlation). However, there was considerable overlap in percent muscle lipid for each visceral fat ranking, so the muscle lipid assay provides better data.

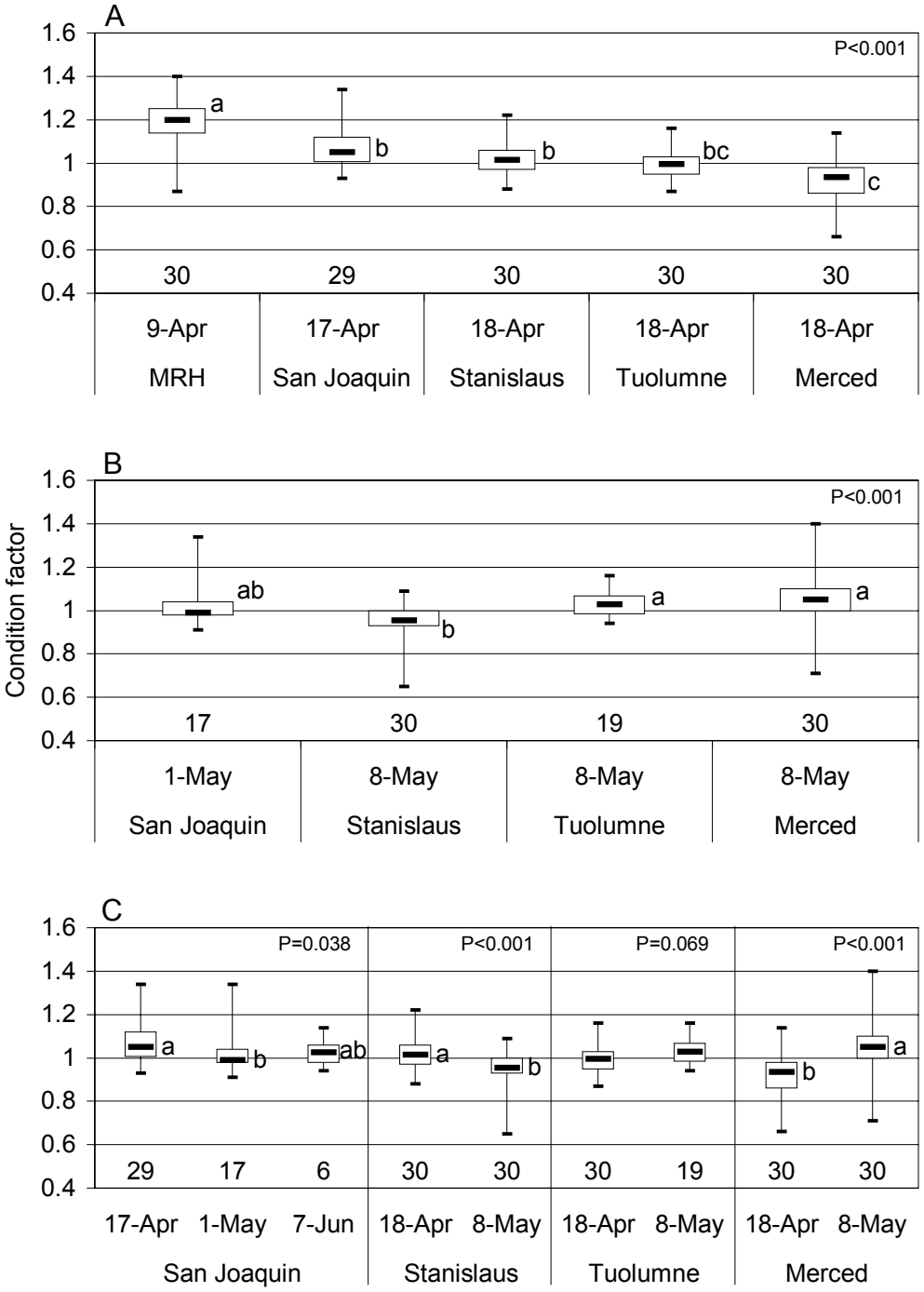

Figure 6. Condition factors (Wt[g] / FL[mm]^3 x 10^5) for juvenile Chinook salmon examined from the Merced River Hatchery (MRH), San Joaquin, Stanislaus, Tuolumne and Merced Rivers. Data is given as Median, 25%-75% (box), range (whiskers), and sample size. Statistical analysis (ANOVA on ranks or Rank Sum Test) is made between sites for April (A) and May (B) and at each site over time (C). Groups that are significantly different (P<0.05) have no letters in common.

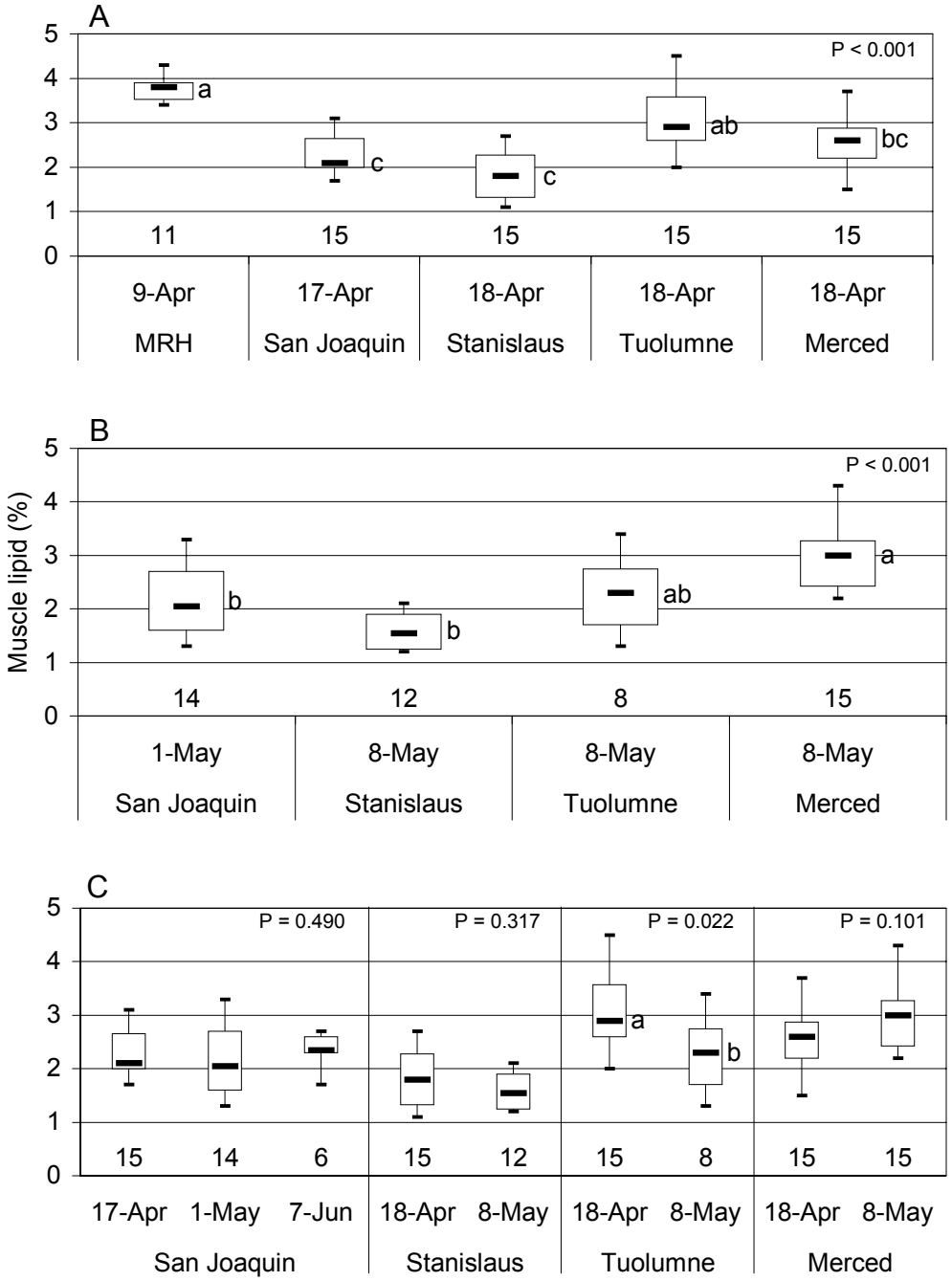

Figure 7. Percent muscle lipid for juvenile Chinook salmon from the San Joaquin, Stanislaus, Tuolumne and Merced Rivers. Data is given as Median, 25%-75% (box), range (whiskers), and sample size. Statistical analysis (ANOVA on ranks or Rank Sum Test) is made between sites for April (A) and May (B) and at each site over time (C). Groups that are significantly different (P<0.05) have no letters in common.

Eosinophilic granular cells (EGC's)

We noted a prevalent layer of EGC's in the lamina propria layer of the intestine of fall Chinook salmon from the San Joaquin River (Figure 8). These immunodefensive cells have been found in many organs, particularly those in direct contact with the environment such as gill, skin, and digestive tract. They are associated with parasitic infections and contain both peroxidase and lysozyme (Sveinbjornsson et al. 1996, Sire and Vernier 1995). Their likely function is to enhance neutrophil transmigration to the site of inflammation (Matsuyama and Iida 2002). While we see these cells only occasionally in juvenile salmon from the Klamath, Trinity and Sacramento Basins, they were of interest because of the high occurrence in San Joaquin basin juvenile Chinook. The EGC band was noted in 70-100% of fish from sites throughout the San Joaquin basin (Figure 9). The development of this layer appeared more stock (genetic or location) related rather than to a contaminant, pathogen or other stimulus. If the trigger was a contaminant or pathogen, it is common to all areas sampled. The utility of the EGC cell layer as an assay is questionable at best. It does not indicate any obvious problem for the smolt, and there is a high degree of subjectivity in the identification of the layer.

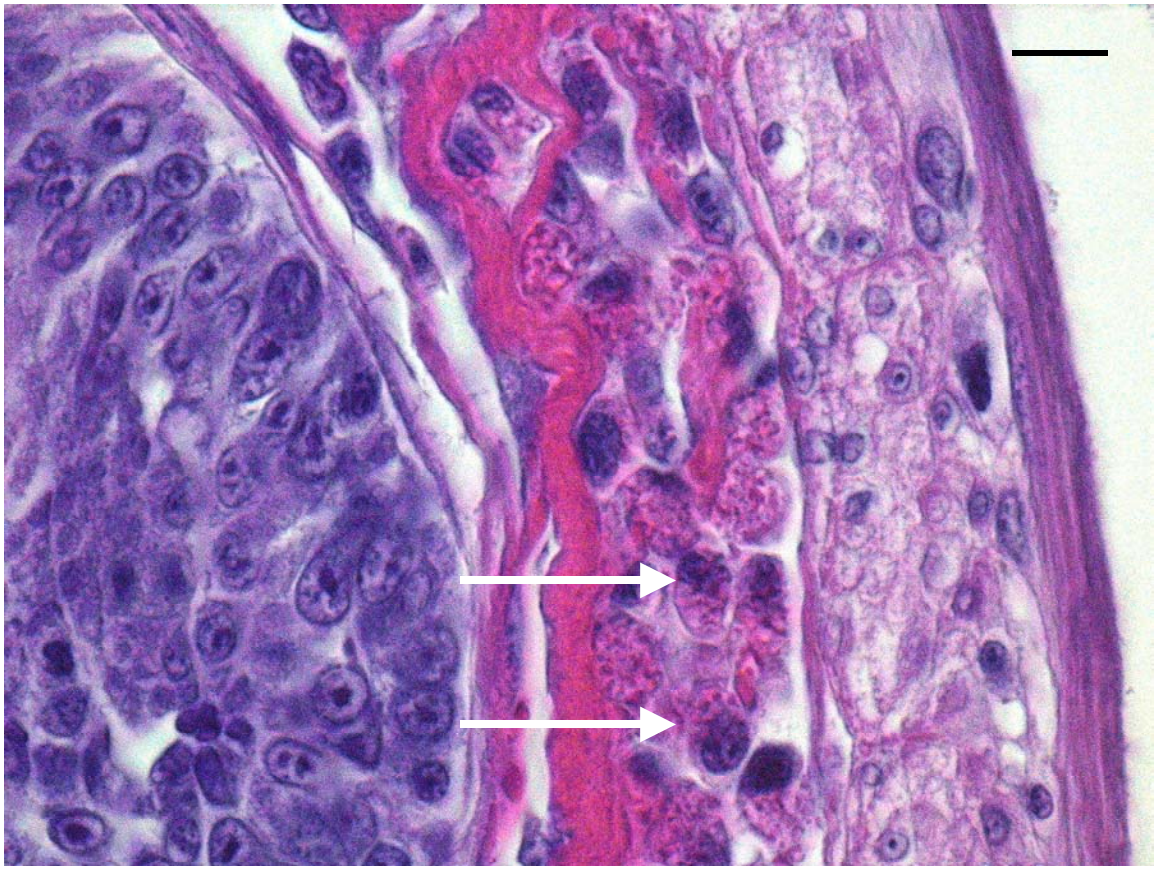

Figure 8. Photomicrograph showing the eosinophilic granular cell layer (EGC rating 3) within the stratum compactum of the pyloric caeca in a Merced River Hatchery Chinook Salmon smolt. Arrows indicate EGC's and bar represents 10 μm. Hematoxylin and eosin stain.

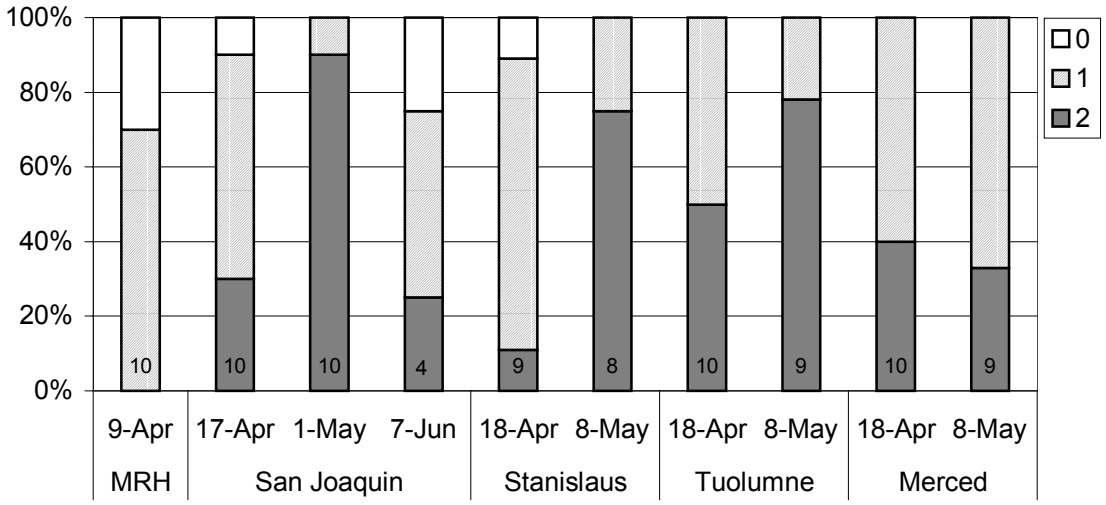

Figure 9. Eosinophilic granular cell (EGC) score indicating the thickness of the EGC layer in the lamina propria of the intestine of Merced River Hatchery (MRH), San Joaquin River, Stanislaus River, Tuolumne River and Merced River juvenile Chinook salmon. Scores range from 0 (no traceable layer present) to 2 (layer greater than 2 cells thick). Number of samples is indicated in the lower portion of each bar.

Gill Na^+, K^+-ATPase (ATPase)

Significant differences in ATPase activity values were observed between sites in April (Figure 10A) but not in May (Figure 10B). There was an expected increase at each site over time (Figure 10C). Natural smolts from the Tuolumne had the highest values in April. By May, smolts from all sample sites were demonstrating elevated ATPase levels. MRH fish ATPase activities during April were similar to natural fish from the San Joaquin, Stanislaus and Merced. The mixed origin smolts from the San Joaquin during June had the highest median ATPase values in the study, but these were not significantly different from natural smolts from the San Joaquin in May. In previous studies peak ATPase activities were during the end of May or beginning of June (Rich and Loudermilk 1991, Nichols et al. 2001). ATPase activity values in this study were not directly compared to work done in the San Joaquin basin during 2000 (Nichols et al. 2001) due to differences in the assay methods and reagents.

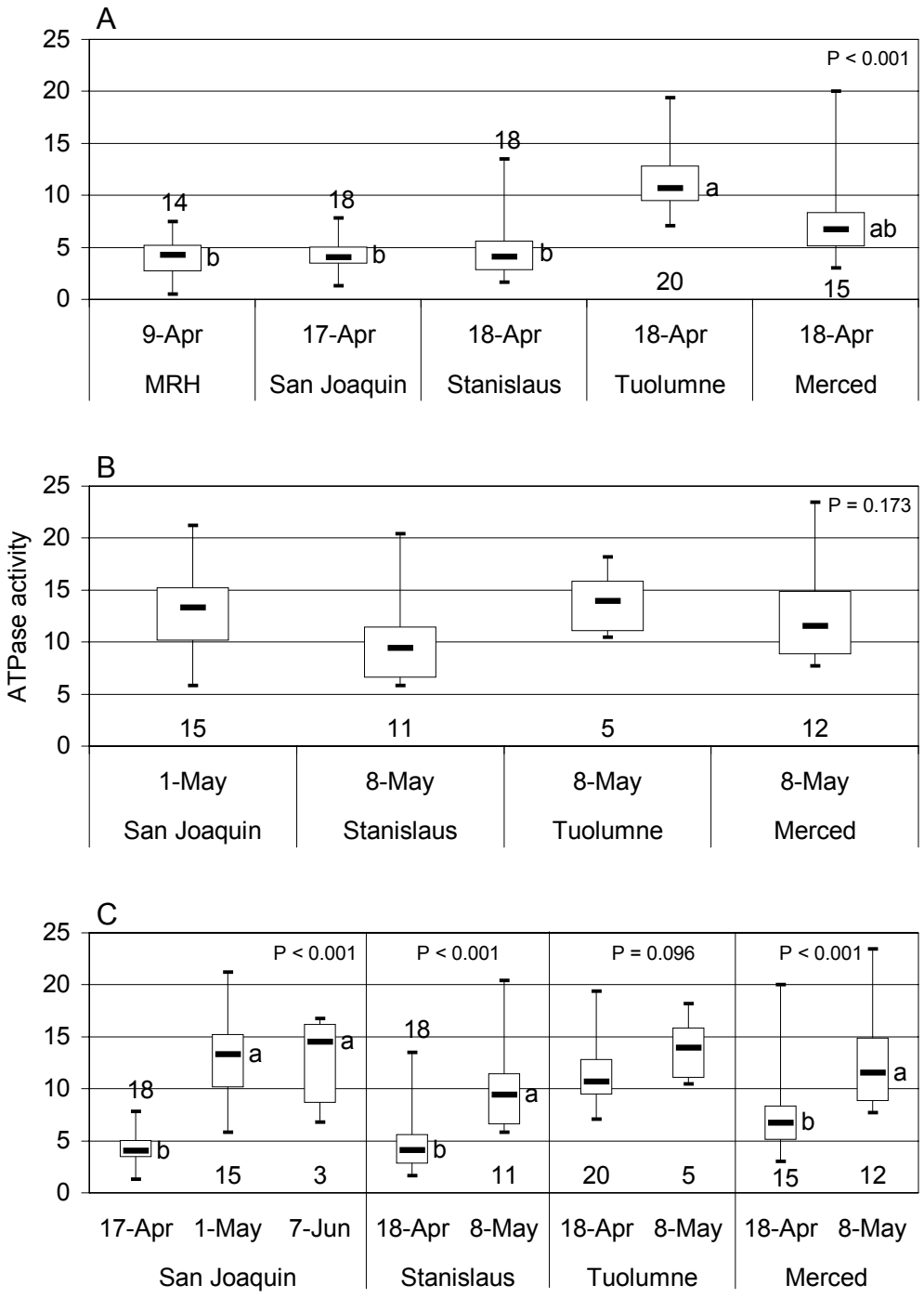

Figure 10. Gill Na+, K+-Adinosine Triphosphatase activity levels (μmol ADP / mg protein / hour) for juvenile Chinook salmon examined from the Merced River Hatchery (MRH), San Joaquin, Stanislaus, Tuolumne and Merced Rivers. Data is given as Median, 25%-75% (box), range (whiskers), and sample size. Statistical analysis (ANOVA on ranks or Rank Sum Test) is made between sites for April (A) and May (B) and at each site over time (C). Groups that are significantly different (P<0.05) have no letters in common.

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Appendix 1. Organ abnormality and visceral fat (Vfat) scores assigned to individual fish based on observation during necropsy

Tissue	Score
Skin	0 = normal scale number, no lesions
	1 = some scale loss, 5 - 20 % of body surface
	2 = focal hemorrhages, scale loss 21 - 40 % of body
	3 = open wound, scale loss > 40 % of body surface
Eye	0 = no abnormalities
	1 = missing 1 eye, diminutive, external abrasion, some opacity
	2 = exophthalmia ("pop-eye"), cataract, bubbles, parasites
	3 = hemorrhage, rupture
Gill	0 = normal condition, color
	1 = pale
	2 = clubbed, frayed, nodules, mild parasite load
	3 = necrotic zones, fungi or bacterial lesions, hemorrhagic
Vfat	0 = no visceral fat on pyloric caeca or peritoneal cavity
	1 = < 50 % coverage of caeca and/or cavity fat vol. < caeca vol.
	2 = >50 % but not covering caeca and/or cavity fat vol. = caeca vol.
	3 = caeca and cavity completely filled with fat, organs obscured by fat